



Investigations into the Development of a Mycelium Biocomposite to Substitute Polystyrene in Packaging Applications

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Abstract

Expanded polystyrene is a versatile synthetic polymer that has a remarkable presence in packaging applications. It is also non-biodegradable in nature which makes them one of the worst solid pollutants that affect the environment. This paper focuses on the possibility of developing an eco-friendly packaging material with mycelium as a binder in a sawdust matrix, and to study the extent up to which these composites are reliable in substituting polystyrene for sustainable packaging applications. The combustion test using a respirable dust sampler has revealed the number of toxic combustion products liberated while burning expanded polystyrene samples. Mycelium fibres were grown from oyster mushrooms, and the analysis of scanning electron microscope images has ensured the growth of mycelium without contamination. Characterization of the newly developed mycelium composite material was done using various techniques, and the results were compared against those of polystyrene. Fourier transform infrared spectroscopy analysis was used to determine the composition and morphology, whereas the thermal behaviour was analysed through tests such as thermogravimetric analysis and differential scanning calorimetry. An X-ray diffraction test was also conducted to identify the compounds present in the samples. The investigation shed light upon the possibility of using the newly developed mycelium bio-composite material to substitute polystyrene as a packaging material.

Keywords Mycelium · Mushroom · Polystyrene · Sustainable packaging · Biocomposites

1 Introduction

Expanded polystyrene (EPS) is a non-biodegradable, petroleum-based polymer which is undoubtedly a frequently used material in the packaging industry. Owing to their excellent shock-absorbing properties, it is a preferred material used for packaging of food, electronic products, and electrical equipment. The low thermal conductivity of EPS accounts for its applicability as an insulation material in the construction industry [1–3]. However, a large portion of EPS used in packaging has a smaller life span of less than one year in terms of its use. A large amount of these packaging materials are single-use products which will eventually turn up

as waste and eventually end up in any landfill site if not properly recycled [4, 5]. Therefore, once EPS is released to the environment as waste, these products will remain in the environment for a very long time due to their resistance against natural decomposition. The manufacturing of polystyrene also has a detrimental environmental impact in terms of energy consumption and toxic gas concentrations [5, 6].

Improper handling of this waste often leads to huge piles of garbage catching fire in the landfill sites and this burning of polystyrene will result in the liberation of toxic combustion products which makes it an environmentally hazardous synthetic polymer. Considering these facts, a biodegradable alternative for polystyrene that can match the performance of polystyrene shall prove to be a great leap forward to a sustainable world [3, 5].

Also, reports show that these adjuvants contain compounds that may adversely affect human health. It is claimed that 50% of plastic products, including polystyrene products, plastic sacks, are intended to be disposable [7]. This global figure has increased by an average rate of 9% from 1950 to a peak of 5 million tons in 2008. Future demand and use of

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polystyrene products remain to expand in developing and emerging countries [8]. A lack of proper waste management in place has increased the EPS waste to the backlog of plastic wastes that are already in existence. Even though EPS can be recycled, the waste management methods adopted in several countries will eventually result in the landfill and thus the development of newer methods to substitute EPS has an inherent ecological value [3]

Mycelium is a quickly developing vegetative component of a fungus that is a secure, sustainable, organic, and green material that develops in a mass twisted fibre, attached to the substrate on which it develops and can originate primarily from biological or agricultural wastes. Mycelium as a material has high biological degradation and good mechanical strength [9–12]. Figure 1 shows the growth of mycelium fibres in sawdust substrate.

Mycelium (Mushroom roots) can be cultivated in a mould to create distinct forms of objects and rapidly develop into a thick substance. Once the required density and structure have been reached, the fabric is dehydrated to prevent further growth. After its useful life as a packaging material, mycelium-based products can be decomposed within a few weeks. Large-scale production of this material will bring down the cost, and they are easier to biodegrade than recycling [13, 14].

The mycelium-based biocomposite materials have the potential to replace less environmentally friendly materials like wood composites or bioplastics. These materials can find applications in interior furnishing of residential apartments or commercial complexes where the fire-resistance and acoustic and thermal insulation properties are crucial. Several studies have reported ongoing investigations to characterise these properties, while there has been only limited research reported on the development and application of mycelium-based composite as an alternative to EPS [15, 16].

This research focuses on the development of a renewable and biodegradable material suitable for packaging applications. The possibility of using mycelium to produce



Fig. 1 Mycelium fibres grown in sawdust

biodegradable materials that have potential properties for the replacement of EPS in packaging applications is being investigated. Material characterization of the biocomposite has been done through thermal stability analysis, morphological analysis, or by determining physical properties and toxicity analysis. The obtained results are compared with the properties of polystyrene.

2 Materials and Methods

2.1 Materials Used

Pleurotus ostreatus species oyster mushroom is used for the development of mycelium. The growth of mycelium is enhanced by using a medium of potato dextrose agar (PDA), (HI media MH096). The biocomposite material is prepared in a matrix of sawdust. Sawdust with fine particles is used for preparing the composite since the particle size of sawdust directly affects the binding of mycelium. The prepared biocomposite is chemically sterilized, and the excess moisture content is removed from it.

2.2 Manufacturing

The manufacturing of the mycelium-based composite can be split into two phases. During the first phase, the mycelium/mushroom roots of *Pleurotus ostreatus* species of oyster mushroom are grown and the second phase is the preparation of the composite sample.

2.2.1 Development of Mycelium

A sample size of 3.9 g Potato dextrose agar (PDA) medium is added to 100 ml of distilled water, and the mixture is steam sterilized in an autoclave at a pressure of 1.03×10^5 N/m² and a temperature of 121 °C for about 15 min. After sterilization, PDA is cooled to 45 °C and poured into sterile Petri plates and allowed to solidify. The fruiting body of the mushroom was sliced into tiny parts using sterile forceps and washed thrice with sterile distilled water. With the help of sterile forceps, the mushroom was kept at the base of the PDA and kept for incubation at 25 °C for about 5 days. The temperature range is important for the proper growth of mycelium and is between 24 and 27 °C [17–21]. Figure 2 shows the various samples of mycelium fungus grown in the laboratory.

2.2.2 Manufacturing of Composite

The sawdust is chemically sterilized and has been drained. A major portion of the formed mycelium is separated from the Petri plate and placed in a container (30 × 10 cm cylindrical

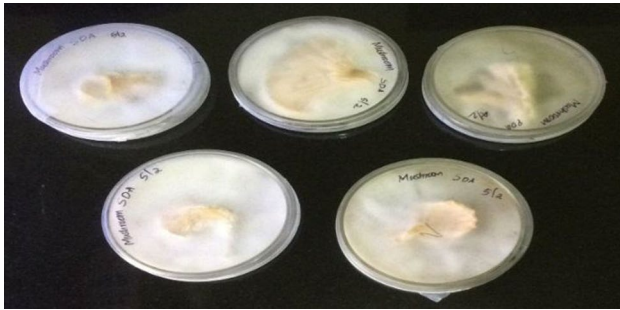


Fig. 2 Mycelium fungus grown in the laboratory



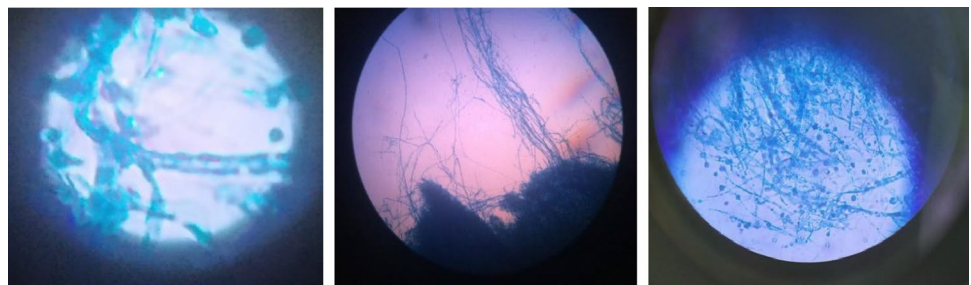
Fig. 3 Mycelium-sawdust composite samples

shape) along with the sterilized sawdust and provides sources of nutrition for the further development of mycelium. This container was kept at a temperature of 25 °C and relative humidity of 80%. It requires 8–10 days for the mycelium to attach entirely to the substrate. This product is then transferred to the mould so that the bonds become closer and form a stable structure. Figure 3 shows the mycelium-sawdust bio-composite samples developed from sterilized sawdust and mycelium fungus grown in the laboratory.

2.3 Equipment Used

The mycelium bio-composite material was characterized and compared against polystyrene using various techniques like

Fig. 4 Microscopic images of the growth of fungal mycelium



the Toxicity test using a Respirable Dust Sampler, Fourier transform infrared spectroscopy, thermogravimetric analysis, differential scanning calorimetry, scanning electron microscopy, energy-dispersive spectroscopy, and X-ray diffraction analysis. Fourier transform infrared spectroscopy (FTIR) analysis is used to determine the composition and morphology, whereas the thermal behaviour is analysed through tests such as thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC). Scanning electron microscopy (SEM) analysis gives an idea about porosity in the material, and the energy-dispersive spectrum (EDS) gives the details of elements present in the specimen. An X-ray diffraction (XRD) test is also conducted to identify the compounds present in the samples.

3 Results and Discussion

3.1 Characterization of Mycelium

The microscopic images of mycelium undergrowth are shown in Fig. 4. These pictures are drawn at a seven-day interval. After the mushroom is moved to the dextrose agar, the first image is taken on the seventh day. The image is not evident and is subject to contamination, i.e. growth of non-mycelium external fungi. By dividing the contaminated parts, the mushroom is sub-cultivated in a fresh tray. After the mushroom subculture, the second image is taken on the seventh day. The picture's small sections depict Mycelia's development. Besides, the third image shows the ongoing growth of mycelium without contamination.

3.2 FTIR Spectrum of Mycelium

The infrared spectra for mycelium were recorded in an FTIR spectrophotometer (Thermofisher, Nicolet 7000) as per ASTM E1421 with a transmittance range of scan from 4000 to 500 cm^{-1} , and Fig. 5 depicts the FTIR spectrum of fungal mycelium. Even though all the samples were dried using a laminar flow chamber, a bond of the water molecule is present in the biomolecules of the sample even

after the dehydration cycle. The presence of water molecules in the sample is evident from the FTIR spectrum, and the broadband between 3000 and 3650 cm^{-1} is partly due to the presence of OH group in the sample and partly due to the absorption of water. The 2161 cm^{-1} band reflects an alkyne functional group corresponding to the $\text{C}\equiv\text{C}$ stretching modes. The band at 1635 cm^{-1} is contributing to $\text{C}=\text{C}$ stretching or $\text{N}-\text{H}$ bending. The intermediate range at 1376.82 is also owing to the twisting of $\text{O}-\text{H}$. A broad absorption band dominates the spectrum in the lower region at 1034.80 cm^{-1} . This peak arises mainly from carbohydrate and nucleic acid vibrations [22, 23].

3.3 Characterization of Biocomposite and Polystyrene

3.3.1 SEM with EDS

SEM analysis is used to characterize the morphology and surface topography of the samples, while EDS helps to find

the composition of different elements in the sample [24]. Both SEM and EDS analyses were performed using a field emission scanning electron microscope (FESEM) (Carl Zeiss microscopy Ltd & ZEISS Sigma 500). Figure 6a shows the SEM image of polystyrene at a magnification of 500X, and Fig. 6b shows the EDS spectrum for the polystyrene specimen. Based on the spectrum, polystyrene is found to have a porous microstructure. From the spectrum of EDS analysis, the elements that can be found on polystyrene are calcium (Ca), carbon (C), oxygen (O), magnesium (Mg), aluminium (Al), silicon (Si), and potassium (K).

Figure 7a shows the SEM image of the mycelium biocomposite specimen at a magnification of 500X which shows a loosely packed sawdust matrix with mycelium binder. This accounts to the lightweight characteristics of the mycelium biocomposite specimen along with porosity. Figure 7b shows the energy-dispersive spectrum from which the details of the elements present in the specimen can be identified. The main elements present in the sample are potassium (K),

Fig. 5 FTIR spectrum of fungal mycelium without contamination

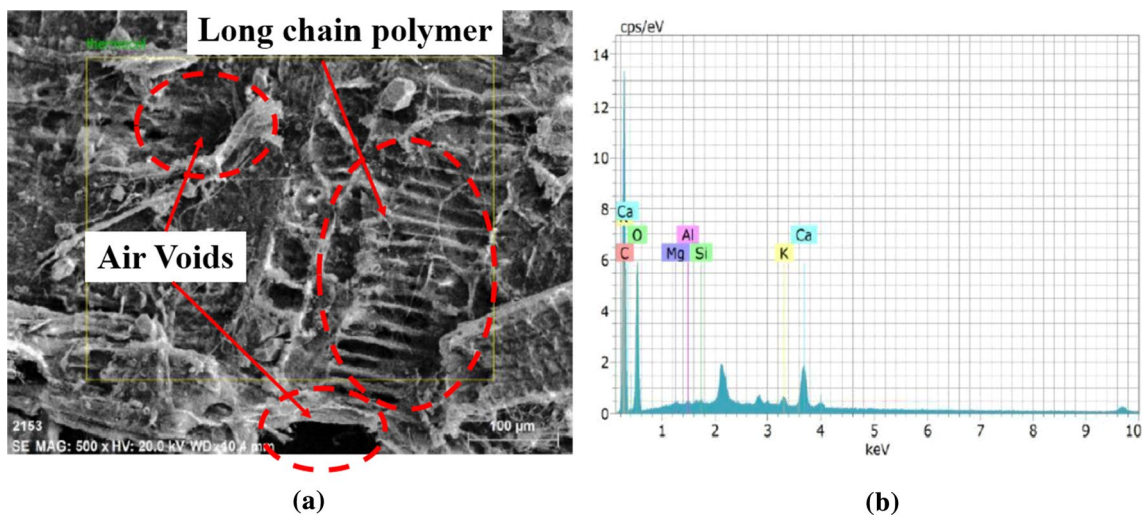
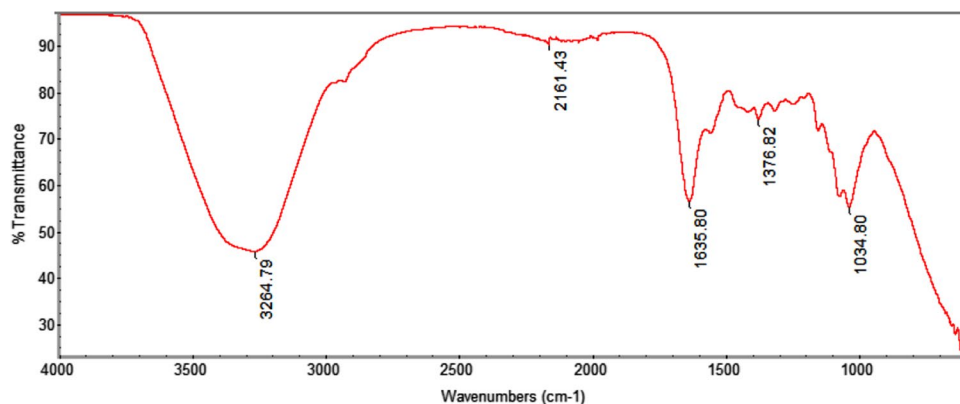


Fig. 6 **a** SEM image and **b** EDS spectrum of polystyrene



carbon (C), oxygen (O), magnesium (Mg), aluminium (Al), and silicon (Si).

The SEM image of mycelium biocomposite material reveals a network-like microstructure in the matrix with a fine filament distribution, while that of the polystyrene samples shows a plate-like structure. It is observed that the mycelium biocomposite material has an open cell structure which provides a cushioning effect to the material which is very significant for packaging applications. The SEM image indicates that the mycelium biocomposite material has a well-defined structure when compared to polystyrene particles which exhibited a long polymeric chain structure. A continuous orientation throughout the matrix is observed through the SEM image of mycelium biocomposite materials which also indicates that the physical properties of the material are consistent throughout the material. The polystyrene particles are more densely packed than the mycelium biocomposite material which is evident from the higher density of polystyrene samples.

3.3.2 FTIR Spectrum Analysis

The FTIR spectrum of polystyrene samples is shown in Fig. 8a. The absorbance peaks are located along with a wide range of wavenumber spectra scale. The peak at 3024.74 cm^{-1} is for aromatic C–H stretching vibrations, and the next peak is for C–H stretching at 2919.44 cm^{-1} which occurred due to the presence of some impurities on the sample. The next three peaks that are located at 1600.74 , 1492.13 , and 1451.49 cm^{-1} , respectively, indicate aromatic C–H bond stretching vibration. The peaks at 1180.78 , 1067.95 , 1027.64 , 905.78 , and 841.79 cm^{-1} correspond to the aromatic C–H deformation vibration, while the peak at

753.64 cm^{-1} indicates the CH_3 twisting deformation or rocking deformation.

The result of the sample is qualitative and confirmed that the vibration positions belong to polystyrene which is in line with the reported literature. The weak and broad band at 3329.41 cm^{-1} in the FTIR spectrum of biocomposite specimens indicates the O–H stretching of alcohol. The bands at 2161.15 cm^{-1} , 2050.35 cm^{-1} , and 1980.06 cm^{-1} show C≡C stretching of alkynes and C–H bending of an aromatic compound. Weak bands at 1628.43 cm^{-1} , 1505.58 cm^{-1} , 1369.03 cm^{-1} , and 1236.33 cm^{-1} show C=C stretching and N–H bending of amines. The strong and sharp band at 1035.04 represents S=O stretching of sulphoxide [20, 21].

3.3.3 Thermogravimetric Analysis

A TGA test was performed to analyse the thermal stability of biocomposite samples over some time for an increase in temperature at a constant rate of heating [24, 25]. The analysis was carried out on both polystyrene and biocomposite samples at a heating rate of $10\text{ }^\circ\text{C}/\text{min}$ with air as the atmosphere using a thermogravimetric analyser (NETZSCH & STA 449 F3 Jupiter) as per ASTM D3418. The samples experienced thermal decomposition in a programmed temperature range of $20\text{--}550\text{ }^\circ\text{C}$ and continuous weight loss and temperature were recorded and analysed. Figure 9a shows the TGA spectrum of polystyrene specimens. The results show that the polystyrene undergoes thermal degradation beginning at $390\text{ }^\circ\text{C}$ and with a total mass loss of 95%. The temperature from 390 to $420\text{ }^\circ\text{C}$ shows the decomposition of the polystyrene. Even if the temperature exceeds $420\text{ }^\circ\text{C}$, the mass of the sample remains almost the same. This is because that the last stage consists of the inert inorganic residues of ash or other filler

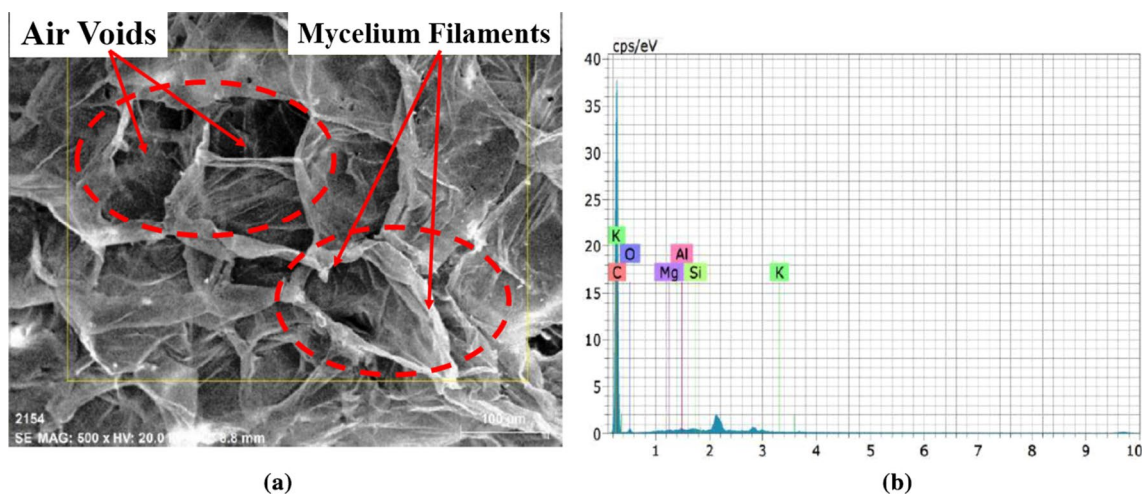
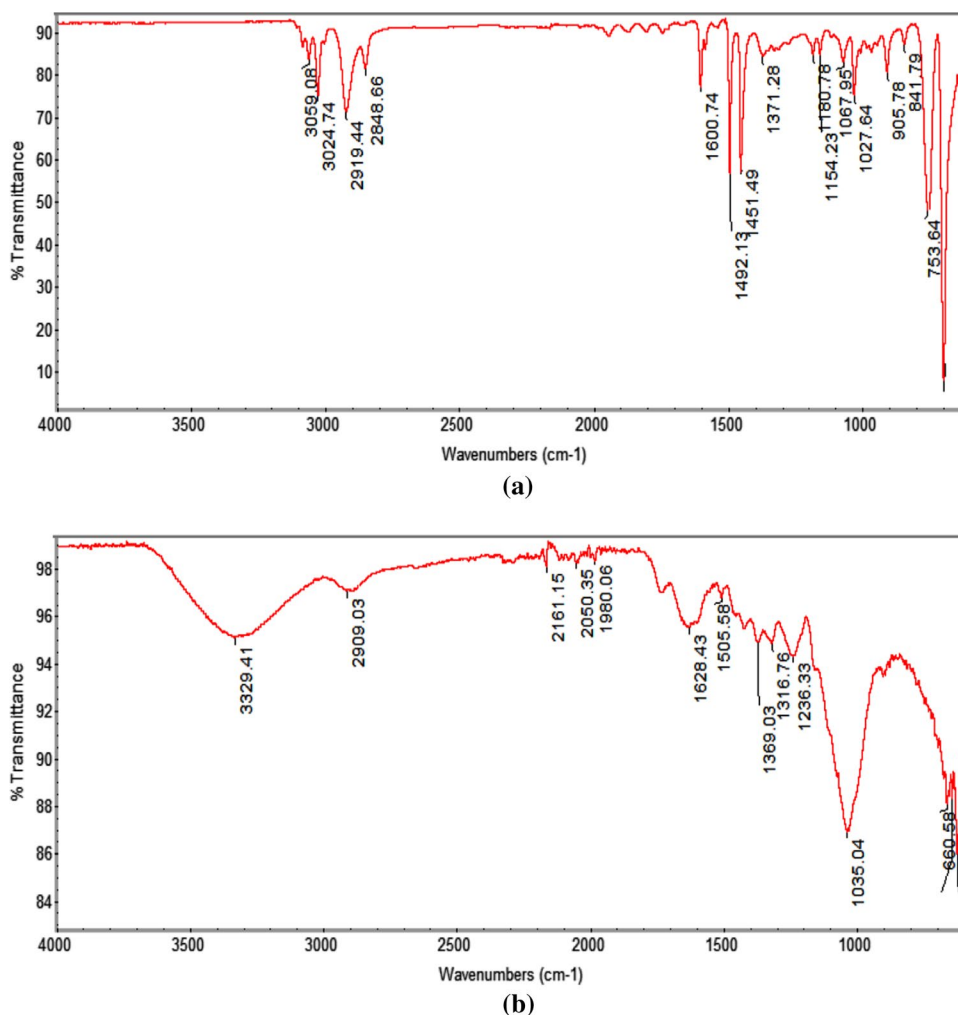


Fig. 7 a SEM image of mycelium biocomposite specimens, b EDS spectrum of mycelium biocomposite specimens

Fig. 8 **a** FTIR spectrum of polystyrene, **b** FTIR spectrum of mycelium biocomposite specimens



materials. The final stage of the specimen is at 547.8 °C with a residue mass of 18.91%.

Figure 9b shows the TGA spectrum of the sawdust-mycelium composite specimen. Almost 10 mg of the composite specimen is taken for the TGA analysis, and it is heated in a defined atmosphere. The graph can be divided mainly into three sections. One is the loss of volatile components such as moisture, solvents, and monomers. Section two represents decomposition. And the third one is the inert inorganic residue of ash, filters, etc. The composite specimen undergoes multi-decomposition as the TGA spectrum shows several steps. From the final stage of the TGA test, the mass of the sawdust-mycelium composite is reduced to 18.23% at a temperature of 547.6 °C.

The thermal degradation of EPS starts from 390 °C, and that of the biocomposite is around 280 °C. It indicates that less energy is required for the mycelium-sawdust composite for the phase transition when compared to the polystyrene. The biocomposite specimen shows a multi-decomposition spectrum in the TGA analysis. The complete transformation

of the composite will reach around 500 °C, but in the case of polystyrene, the complete transformation occurs at 420 °C. Thus, we can conclude that the mycelium-sawdust composite is more stable than the polystyrene.

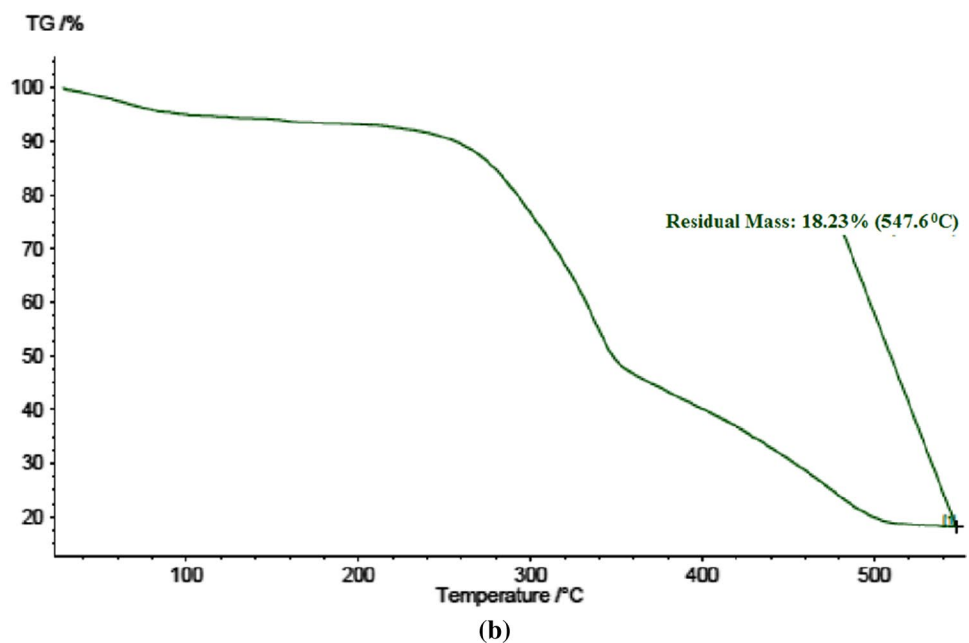
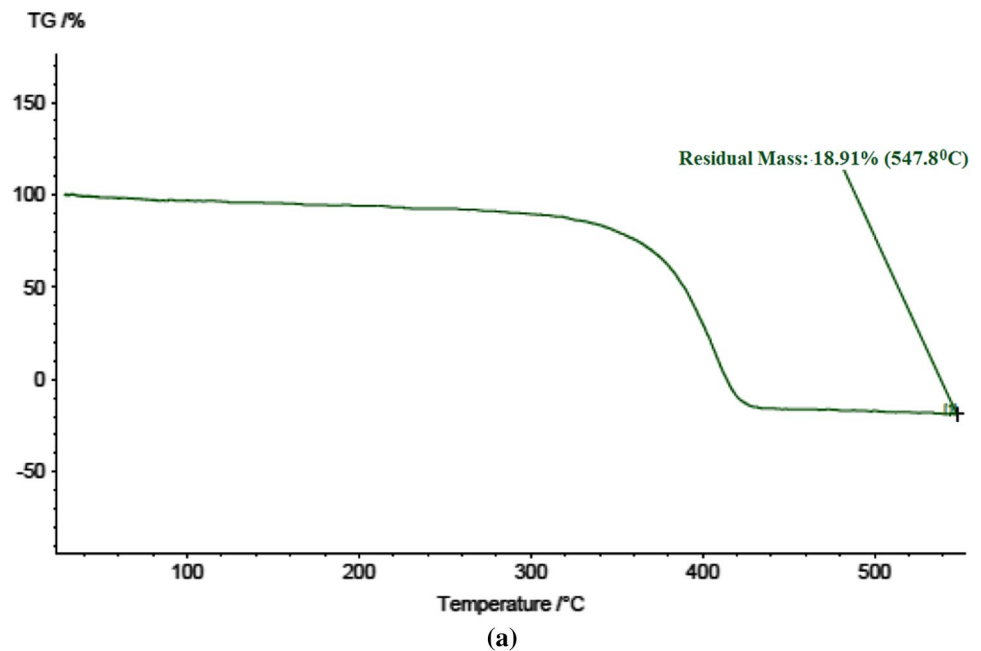
3.3.4 Differential Scanning Calorimetry

DSC analysis was carried out on both polystyrene and biocomposite samples at a heating rate of 10 °C/min using a differential scanning calorimeter (NETZSCH & STA 449 F3 Jupiter) as per ASTM E793 with air as the atmosphere. All measurements of each sample were made in a programmed temperature range of 0–550 °C. Figure 10a shows the DSC spectrum of polystyrene which clearly shows an exothermic reaction, with a peak at 418.5 °C. The transition is starting from 405.4 °C and ends at 429.7 °C. The area under the curve is around 92 J/g, which gives the amount of heat absorbed during the transition.

DSC analysis is done on the sawdust-mycelium biocomposite, and the corresponding spectrum is shown in Fig. 10b.



Fig. 9 **a** TGA of polystyrene. **b** TGA of the mycelium biocomposite specimen

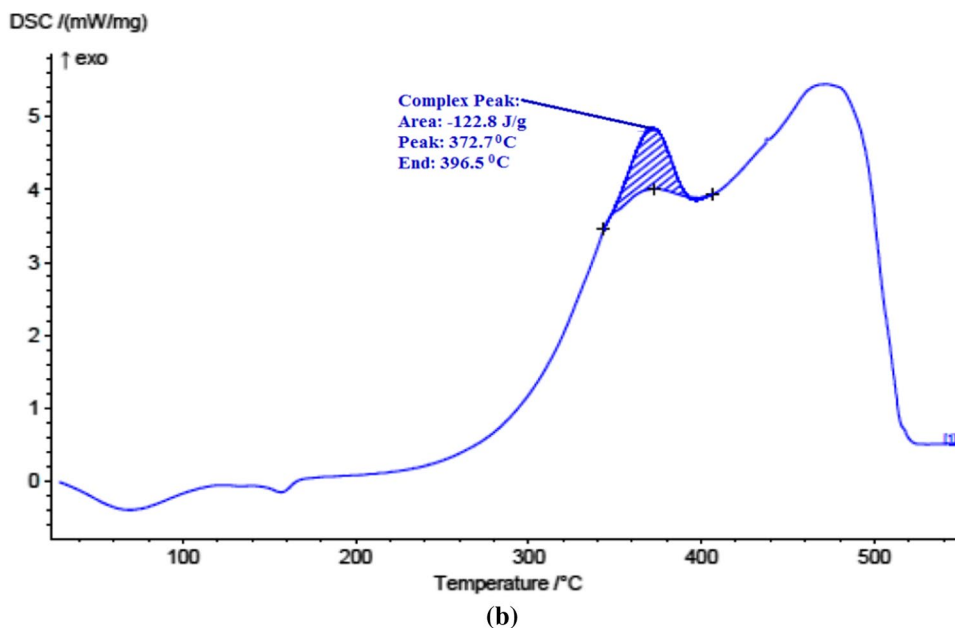
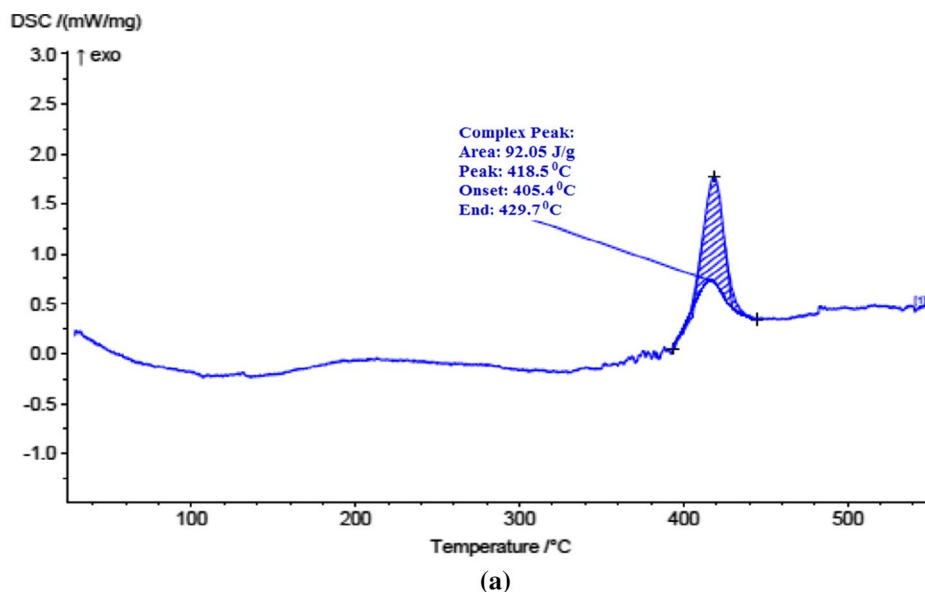


The transition stage is represented by the shaded region. It starts at a temperature around 350 °C and ends at 396.5 °C. The peak value is at a temperature of 372.7 °C. Since the area under the curve is given a negative value, the process might be an endothermic reaction. And the value of heat now is about – 122.8 J/g. Another peak is also visible in the DSC spectrum of the sawdust-mycelium specimen. These multiple peaks in the spectrum might be due to the two different morphologies in the specimen.

3.3.5 X-ray Diffraction

X-ray diffraction analysis was done using an X-ray diffractometer (Bruker AXS, D8 Advance Series 1). The XRD spectrum of polystyrene specimens confirms that the main compound in the polystyrene specimen is lithiophorite ($\text{H}_{42}\text{Al}_{14}\text{Li}_6\text{Mn}_{21}\text{O}_{84}$) having the minerals of lithiophorite. The compound has a hexagonal crystal structure. The peak in intensity in the graph occurred when the mineral contains lattice planes with d-spaces appropriate to diffract X-rays at that value of θ .

Fig. 10 **a** DSC analysis of polystyrene, **b** DSC analysis of the mycelium biocomposite specimen



The XRD analysis is performed to identify the underlying compounds and their crystalline structure in the sample. Two of the main compounds and their crystalline structures are identified from the XRD spectrum of the biocomposite specimen. They are cellulose-I β with a chemical formula of $(C_6H_{10}O_5)_n$ and 1-(4-ethoxyphenyl) bicyclo (3.3.0)-5-aza-1-boro 2,8-dioxaoctane ($C_{12}H_{18}BNO_3$). Here the peak intensity in the spectrum occurred when the minerals contain lattice planes with d-spaces appropriate to diffract X-rays at that value of θ . The identified compounds were found to have a monoclinic crystal structure.

3.4 Toxicity Test

The burning of polystyrene results in the emission of hazardous gases and the release of particulate matter concentration to the atmosphere. A toxicity test was performed using a Respirable dust sampler (Envirotech, APM 460 NL) to determine the extent of particulate matter concentration and hazardous gases emitted as a result of burning polystyrene and the newly developed mycelium biocomposite specimens. 800 g of both specimens was used to conduct the test. The presence of gases like nitrogen dioxide, carbon monoxide, and sulphur dioxide was identified and quantified. The amount of particulate matter released into the atmosphere



Table 1 Results of the combustion test

S. no	Combustion products in $\mu\text{g}/\text{m}^3$	Expanded polystyrene	Mycelium biocomposite
1	Sulphur dioxide	95.59	167.87
2	Nitrogen dioxide	89.78	43.80
3	Particulate matter	1643.0	153.31
4	Carbon monoxide	49	92

is also found. Table 1 shows the consolidated results of the combustion test.

Since the biocomposite is made of mycelium and sawdust, the burning of which releases more carbon monoxide and sulphur dioxide than EPS. However, the amount of particulate matter released to the atmosphere is very less for the biocomposite than that of polystyrene. Nitrogen dioxide emissions from polystyrene are nearly double that of the biocomposite material. Though mycelium biocomposite materials release significant amounts of sulphur dioxide and carbon monoxide while burning, the instances of mycelium material catching a fire are very rare. Incineration is not a preferred method of waste disposal for both EPS and mycelium biocomposite; however, polystyrene waste is more prone to accidental fires in a landfill site as they are non-biodegradable. Mycelium-based biocomposite specimens tend to biodegrade faster in extreme weather conditions, and thus the chances of it being prone to fire in a landfill site become less [11, 18].

3.5 Physical Properties

Physical properties like density and moisture content of the mycelium-sawdust biocomposite is identified, and the analysis revealed that the prepared specimen is homogeneous in nature.

3.5.1 Density of the Sample

The bulk density of mycelium biocomposite is calculated using the formula.

$$\text{Density}(\rho) = \frac{M}{(L \times w \times t)} \frac{\text{g}}{\text{cm}^3} \quad (1)$$

Since bulk density and porosity are related to each other, comparing the values of density can give an idea about the porosity of the samples [25]. The average value of bulk density for mycelium-sawdust biocomposite was found to be $0.1785 \text{ g}/\text{cm}^3$, and that of polystyrene as $1.04 \text{ g}/\text{cm}^3$. A total of three specimens were tested, and the value was the same for each test which shows that the composite specimen has uniform density throughout the material. The

newly developed mycelium-based biocomposite material is far lighter than the densely packed EPS samples as evident from the SEM images. Large air voids present in the mycelium biocomposite can be observed from the SEM micrograph, whereas the air voids present in EPS samples were smaller and uniformly distributed throughout the samples. These results are in accordance with the previous results that reported various mycelium-based composites as light weight composites reporting similar values of density [11]. It is also quite obvious due to the fact that the mycelium biocomposite materials were not manufactured and rather they were biologically grown without applying compressive forces. These materials if subjected to compression may exhibit higher density and may witness an overall shift in its physical and mechanical properties. The effect of densification in these newly developed materials is not yet studied or reported and would be an interesting case to begin with.

3.5.2 Moisture Content

The amount of water contained in a material, commonly known as moisture content, is expressed as a ratio in terms of percentage [26]. The moisture content in a given sample can be calculated using the following formula,

$$\text{Moisture content} = \frac{(M1 - M2)}{M1} \times 100 \text{ in } \% \quad (2)$$

The moisture content present in the mycelium biocomposite samples was found to be around 30%, while polystyrene had a near-zero percentage of water retention even after it was immersed in water for 24hrs. These results have a significant impact when the proposed primary application of the newly developed material is in the packaging industry. The material has to be made moisture resistant before it can be recommended as a substitute to EPS. These results also indicate that the mycelium biocomposite material is biodegradable as well. Applying a thin film of lamination over the surface of the biocomposite may greatly increase the moisture resistance and may increase the shelf life, but it may reduce the biodegradability of the material. More research is required on this aspect of the material weighing the various factors before an unambiguous conclusion can be drawn.

4 Conclusion

The mycelium biocomposite samples prepared could be an effective substitute to petroleum-based polymers like expanded polystyrene. The mycelium biocomposite samples can be prepared as a compact, dense material and they exhibit promising results when subjected to the various

experimental tests. The TGA and DSC analysis show that the mycelium-sawdust biocomposite specimens are more stable when compared to expanded polystyrene. Results of toxicity tests indicate that expanded polystyrene releases more toxic substances when subjected to burning than the burning of mycelium biocomposite samples. These results are in agreement with the previous results which reports the flame resistant nature of mycelium-based composites owing to high char residue and release of water vapour [24]. The average bulk density for the mycelium biocomposite samples are found to be very less when compared to the density of expanded polystyrene samples. Therefore, it can be inferred that the mycelium biocomposite specimens are more porous than the expanded polystyrene samples. Mycelium biocomposite samples are also biodegradable in nature since the primary raw materials used for manufacturing of the mycelium biocomposite samples are made of biodegradable materials like mushroom roots and sawdust. The biodegradable nature of mycelium biocomposite samples significantly influences the selection of this material to substitute expanded polystyrene in packaging applications. When sustainable materials are seen as a strategy to reduce environmental pollution and considering the fact that these renewable materials require only minimum energy for their production, the proposed mycelium biocomposite material is a strong contender to polystyrene in packaging applications.

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